Estriol, Unconjugated, Serum
#81711

Clinical

Estrogens are involved in development and maintenance of the female phenotype, germ cell maturation, and pregnancy. They also are important in many other nongender-specific functions in men and women. These include growth, nervous system maturation, bone metabolism, and endothelial responsiveness.

There are 3 major biologically active estrogens in humans: estrone (E1), estradiol (E2), and estriol (E3). Like all members of the steroid hormone family, they diffuse into cells and bind to specific nuclear receptors, which in turn alter gene transcription in a tissue-specific manner. E2 is the most potent natural human estrogen, closely followed by E1, while E3 possess only 20% of E2’s affinity for the estrogen receptor. In men and nonpregnant women, E1 and E2 are formed from the androgenic steroids androstenedione and testosterone, respectively. E3 is derived largely through conversion of E2, and to a lesser degree from 16a-metabolites of E1. E2 and E1 can also be converted into each other, and both can be inactivated via hydroxylation and conjugation.

During pregnancy E3 becomes the dominant estrogen. The fetal adrenal gland secretes dehydroepiandrosterone-sulfate (DHEAS), which is converted to E3 in the placenta and diffuses into the maternal circulation. The half-life of unconjugated E3 (uE3) in the maternal blood system is 20-30 minutes, since the maternal liver quickly conjugates E3 to make it more water soluble for urinary excretion. E3 levels increase throughout the course of pregnancy, peaking at term.

Measurement of serum E2 and E1 levels is an integral part of assessment of reproductive function in females, and also has applications in both men and women in osteoporosis risk assessment and monitoring of female hormone replacement therapy.

By contrast, with the exception of epidemiological studies assessing breast cancer risk and other scientific studies, the main value of E3 measurements is in the diagnosis of maternal-fetal diseases. In those settings, measurement of serum uE3 levels plays a major role.

Decreased 2nd trimester uE3 has been shown to be a marker for Down and Trisomy 18 syndromes. It also is low in cases of gross neural tube defects such as anencephaly. Based on these observations, uE3 has become a part of multiple marker prenatal biochemical screening, together with alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and inhibin-alpha (inhibin-α) measurements (#81149 Alpha-Fetoprotein, Four Marker Screen, Maternal Serum, [Quad test]). Low levels of uE3 also have been associated with pregnancy loss, Smith-Lemli-Opitz syndrome (defect in cholesterol biosynthesis), X-linked ichthyosis and contiguous gene syndrome (placental sulfatase deficiency disorders), aromatase deficiency, and primary or secondary fetal adrenal insufficiency.

High levels of uE3, or sudden increases in maternal uE3 levels, are a marker of pending labor. The rise occurs approximately 4 weeks before onset of labor. Since uE3 has been shown to be more accurate than clinical assessment in predicting labor onset, there is increasing interest in its use in assessment of preterm labor risk. In this setting, serial uE3 assessment might be necessary and, therefore, salivary uE3 measurements, which mirror serum levels, are often preferred because of their greater patient convenience.

High maternal serum uE3 levels may also be occasionally observed in various forms of congenital adrenal hyperplasia.

Useful For

• As part of #81149 Alpha-Fetoprotein, Four Marker Screen, Maternal Serum, (Quad test), in biochemical 2nd trimester or integrated 1st-2nd trimester screening for Down syndrome and Trisomy 18 syndrome
• As a marker of fetal demise

(Continued on back)
Useful For (Continued)

- As an element in the prenatal diagnosis of disorders of fetal steroid metabolism, including Smith-Lemli-Opitz syndrome, X-linked ichthyosis and contiguous gene syndrome (placental sulfatase deficiency disorders), aromatase deficiency, primary or secondary fetal adrenal insufficiency, and various forms of congenital adrenal hyperplasia.
- In the assessment of preterm labor risk.
- In conjunction with measurement of E1, E2, and various metabolites in epidemiological studies of breast cancer risk.
- In the context of other basic scientific and clinical studies assessing estrogen metabolism, estrogen and estrogen-like medications, and other endogenous or exogenous factors impacting on estrogen metabolism.

Interpretation

In the context of the Quad test, the measured uE3 value forms part of a complex, multivariate risk calculation formula, using age, gestational stage, and other demographic information in addition to the results of the 4 tested markers for Down syndrome, Trisomy 18 syndrome, and neural tube defect risk calculations.

A serum uE3 below 0.3 multiples of the gestational age median in women who otherwise screen negative in the Quad test, indicates the possibility of fetal demise, Smith-Lemli-Opitz syndrome, X-linked ichthyosis or contiguous gene syndrome, aromatase deficiency, or primary or secondary (including maternal corticosteroid therapy) fetal adrenal insufficiency.

An elevated serum or salivary uE3 above 3 multiples of the gestational age mean, or with an absolute value of more than 2.1 ng/mL, can be an indication of pending labor or fetal congenital adrenal hyperplasia.

In the context of assessment of a patient deemed at risk of preterm labor, a single serum or salivary uE3 measurement within the above cutoffs, has a negative predictive value of labor onset (i.e., labor unlikely within the next 4 weeks) of 98% in low-risk populations, and of 96% in high-risk populations.

Measurements of serum uE3 performed in the context of epidemiological or other basic or clinical scientific studies need to be interpreted in the context of those studies. No overall guidelines can be given.

Cautions

Like any immunoassay, this test can occasionally be subject to nonspecific or specific interferences. We strive to identify and resolve these rare problems whenever they occur, but if the clinical picture is inconsistent with the test results, clinicians should still consider the possibility of a preanalytical or analytical error and contact the Endocrine Laboratory for discussion.

Method

The Access unconjugated estriol assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with estriol-alkaline phosphatase conjugate, paramagnetic particles coated with goat antirabbit IgG, and polyclonal rabbit antiestriol. Estriol in the sample competes with estriol-alkaline phosphatase conjugate for a limited number of binding sites on the specific antiestriol antibody. The resulting antigen:antibody complexes are bound to the capture antibody on the solid phase. Separation in a magnetic field and washing remove materials not bound to the solid phase. A chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of estriol in the sample. (Beckman Coulter Access Unconjugated Estriol Assay Manual)

Specimen Required:
Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 0.5 mL of serum refrigerated or frozen in plastic vial.

Reference Values:
Males: <0.07 ng/mL
Females: <0.08 ng/mL

Analytic Time:
Same day/1 day

Days Set Up:
Monday through Saturday; 9:00 a.m. to 4:00 p.m.

Fee:
$87.40

CPT Code:
82677
Parathyroid hormone (PTH) is produced and secreted by the parathyroid glands located along the posterior aspect of the thyroid gland. The hormone is synthesized as a 115-amino acid precursor (pre-pro-PTH), cleaved to pro-PTH and then to the 84-amino acid molecule, PTH (numbering, by universal convention, starting at the amino-terminus). The precursor forms generally remain within the parathyroid cells.

Secreted PTH undergoes cleavage and metabolism to form carboxyl-terminal fragments (PTH-C), amino-terminal fragments (PTH-N), and midmolecule fragments (PTH-M). Only those portions of the molecule that carry the aminoterminus (i.e., the whole molecule and PTH-N) are biologically active. The active forms have half-lives of approximately 5 minutes. The inactive PTH-C fragments, with half-lives of 24-36 hours, make up >90% of the total circulating PTH and are primarily cleared by the kidneys. In patients with renal failure, they can accumulate to very high levels. Unlike many other PTH immunoassays, the 1-84 Bio-Intact PTH assay measures exclusively the biologically active whole PTH molecule (1-84) and does not cross-react with any PTH fragments. This facilitates the correct diagnosis of parathyroid disorders, particularly in patients with renal failure.

The serum calcium level regulates PTH secretion via negative feedback through the parathyroid calcium sensing receptor (CASR). Decreased calcium levels stimulate PTH release. Secreted PTH interacts with its specific type II G-protein receptor, causing rapid increases in renal tubular reabsorption of calcium and decreased phosphorus reabsorption. It also participates in long-term calciostatic functions by enhancing mobilization of calcium from bone and increasing renal synthesis of 1,25-dihydroxy vitamin D, which, in turn, increases intestinal calcium absorption. In rare inherited syndromes of parathyroid hormone resistance/unresponsiveness and in renal failure, PTH release may not increase serum calcium levels.

Hyperparathyroidism causes hypercalcemia, hypophosphatemia, hypercalcuria, and hyperphosphaturia. Long-term consequences are dehydration, renal stones, hypertension, GI disturbances, osteoporosis, and sometimes neuropsychiatric and neuromuscular problems. Hyperparathyroidism is most commonly primary and caused by parathyroid adenomas. It can also be secondary in response to hypocalcemia or hyperphosphatemia. This is most commonly observed in renal failure. Long-standing secondary hyperparathyroidism can result in tertiary hyperparathyroidism, which represents the secondary development of autonomous parathyroid hypersecretion. Rare cases of mild, benign hyperparathyroidism can be caused by inactivating CASR mutations.

Hypoparathyroidism is most commonly secondary to thyroid surgery, but can also occur on an autoimmune basis, or due to activating CASR mutations. The symptoms of hypoparathyroidism are primarily those of hypocalcemia, with weakness, tetany, and possible optic nerve atrophy.

**Useful For**
- Diagnosis and differential diagnosis of hypercalcemia
- Diagnosis of primary, secondary, and tertiary hyperparathyroidism
- Diagnosis of hypoparathyroidism
- Differential diagnosis of hypercalcemia and renal osteodystrophy

**Interpretation**
- About 90% of the patients with primary hyperparathyroidism have elevated PTH levels. The remaining patients have normal (inappropriate for the elevated calcium level) PTH levels. About 40% of the patients with primary hyperparathyroidism have serum phosphorus levels <2.5 mg/dL and about 80% have serum phosphorus <3.0 mg/dL.
- Some patients with moderate hypercalcemia and equivocal phosphate levels, who have either mild elevations in PTH or (inappropriately) normal PTH levels, may be suffering from familial hypocalciuric hypercalcemia, which is due to inactivating CASR mutations. The renal calcium clearance to creatinine clearance ratio is typically less than 0.01 in these individuals. The condition can be confirmed by CASR gene mutation screening.
- An (appropriately) low PTH level and high phosphorus level in a hypercalcemic patient suggests that the hypercalcemia is not caused by PTH or PTH-like substances.

(Continued on back)
**Interpretation (Continued)**

- An (appropriately) low PTH level with a low phosphorus level in a hypercalcemic patient suggests the diagnosis of paraneoplastic hypercalcemia caused by parathyroid related peptide (PTHRP). PTHRP shares N-terminal homology with PTH and can transactivate the PTH receptor. It can be produced by many different tumor types.
- A low or normal PTH in a patient with hypocalcemia suggests hypoparathyroidism, provided the serum magnesium level is normal. Low magnesium levels inhibit PTH release and action and can mimic hypoparathyroidism.
- Low serum calcium and high PTH levels in a patient with normal renal function suggest resistance to PTH action (pseudohypoparathyroidism type 1a, 1b, 1c, or 2) or, very rarely, bio-ineffective PTH.
- Patients with renal failure, whose serum phosphate levels are normal, but who still have elevated Bio-Intact PTH (1-84) levels, probably suffer from secondary or tertiary hyperparathyroidism. If their corrected or ionized serum calcium is normal or high, then the most likely diagnosis is tertiary hyperparathyroidism.

**Cautions**

- For diagnostic purposes, Bio-Intact PTH (1-84) values should be interpreted with other test results, and the overall clinical presentation and history of the patient.
- Normal reference ranges may vary based on geographical locations of the populations studied.
- Although the PTH-N fragments PTH(1-31) or PTH(1-34) do not cross-react in the assay, as they do not bind to both detection and capture antibodies, they do bind to 1 of the assay’s antibodies. Therefore, if PTH(1-31) or PTH(1-34) are present at very high concentrations, they may bind sufficient antibody to interfere in the assay, resulting in falsely low Bio-Intact PTH (1-84) measurements. There are no known disease states where this could occur. However, this situation could conceivably be seen when a patient is receiving exogenous PTH(1-34). Exercise caution when interpreting PTH levels in these patients.

**References**


**Method**

The Nichols Advantage Bio-Intact (1-84) assay is a 2-site chemiluminescence immunoassay for the measurement of biologically active, whole molecule parathyroid hormone (PTH) in human serum. The patient specimen is incubated simultaneously with a biotinylated-goat polyclonal antibody to PTH, a second goat polyclonal antibody to PTH labeled with an acridinium ester, and streptavidin coated magnetic particles. During the subsequent 30-minute incubation, a sandwich complex is formed by the 2 antibodies and PTH in the patients’ specimen. This complex is captured by the streptavidin-coated particles due to high affinity interaction between biotin and streptavidin. The magnetic particles are washed to remove any unbound components and then transported into a luminometer where a chemiluminescence reaction is initiated. The amount of light generated is directly proportional to the concentration of PTH in the specimen.

(Package Insert: Nichols Advantage Bio-Intact PTH (1-84). Nichols Institute Diagnostics, San Juan Capistrano, CA)

**Specimen Required:** Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down, separate from clot, and send 0.8 mL of serum frozen in plastic vial.

**Reference Values:**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>PTH, 1-84 BIO-INTACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 years</td>
<td>Not established</td>
</tr>
<tr>
<td>≥2 years</td>
<td>10-55 pg/mL</td>
</tr>
<tr>
<td></td>
<td>1.1-5.8 pmol/L</td>
</tr>
</tbody>
</table>

Conversion factor: PTH: pg/mL x 0.1061 = pmol/L (molecular wt = 9424)

The reference range has been generated from healthy US Northern Latitude donors. The upper limit of the reference range is likely to be lower in geographical regions with greater sunlight-exposure. In the presence of normal renal function and hypercalcemia, a PTH, 1-84 Bio-Intact level of >35 pg/mL is highly suggestive of primary hyperparathyroidism.

**Analytic Time:** 1 day
**Days Set Up:** Monday through Saturday; 8 a.m. - 2 p.m.
**Fee:** $201.10
**CPT Code:** 83970
Estrogens, Estrone (E1) + Estradiol (E2), Fractionated, Serum #84230

Profile Information

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Reporting Title</th>
<th>Available Separately</th>
</tr>
</thead>
<tbody>
<tr>
<td>81418</td>
<td>Estrone, S</td>
<td>Yes</td>
</tr>
<tr>
<td>81816</td>
<td>Estradiol, Enhanced, S</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Clinical

Estrogens are involved in development and maintenance of the female phenotype, germ cell maturation, and pregnancy. They also are important for many other, nongender-specific processes, including growth, nervous system maturation, bone metabolism/remodeling, and endothelial responsiveness. The 2 major biologically active estrogens in nonpregnant humans are estrone (E1) and estradiol (E2). A third bioactive estrogen, estriol (E3), is the main pregnancy estrogen, but plays no significant role in nonpregnant women or men.

E2 is produced primarily in ovaries and testes by aromatization of testosterone. Small amounts are produced in the adrenal glands and some peripheral tissues, most notably fat. By contrast, most of the circulating E1 is derived from peripheral aromatization of androstenedione (mainly adrenal). E2 and E1 can be converted into each other, and both can be inactivated via hydroxylation and conjugation. E2 demonstrates 1.25-5 times the biological potency of E1. E2 circulates at 1.5-4 times the concentration of E1 in premenopausal, nonpregnant women. E2 levels in men and postmenopausal women are much lower than in nonpregnant women, while E1 levels differ less, resulting in a reversal of the premenopausal E2:E1 ratio. E2 levels in premenopausal women fluctuate during the menstrual cycle. They are lowest during the early follicular phase. E2 levels then rise gradually until 2-3 days before ovulation, at which stage they start to increase much more rapidly and peak just before the ovulation-inducing LH/FSH surge, at 5-10 times the early follicular levels. This is followed by a modest decline during the ovulatory phase. E2 levels then gradually increase again until the midpoint of the luteal phase and thereafter decline to trough, early follicular levels.

Measurement of serum E2 forms an integral part of the assessment of reproductive function in females, including assessment of infertility, oligo-amenorrhea, and menopausal status. In addition, it is widely used for monitoring ovulation induction, as well as during preparation for in vitro fertilization (IVF). For these applications E2 measurements with modestly sensitive assays suffice. However, extra sensitive E2 assays, simultaneous measurement of E1, or both are needed in a number of other clinical situations. These include inborn errors of sex steroid metabolism, disorders of puberty, estrogen deficiency in men, fracture risk assessment in menopausal women, and increasingly, therapeutic drug monitoring, either in the context of low-dose female hormone replacement therapy or antiestrogen treatment.

Useful For

- This test allows the simultaneous high-sensitivity determination of serum E1 and E2 levels. It is useful in situations requiring either higher sensitivity E2 measurement, or E1 measurement, or both. This includes the following:
  - As part of the diagnosis and work-up of precocious and delayed puberty in females, and, to a lesser degree, males
  - As part of the diagnosis and work-up of suspected disorders of sex steroid metabolism, e.g., aromatase deficiency and 17 alpha-hydroxylase deficiency
  - As an adjunct to clinical assessment, imaging studies, and bone mineral density measurement in the fracture risk assessment of postmenopausal women, and, to a lesser degree, older men
  - Monitoring low-dose female hormone replacement therapy in postmenopausal women
  - Monitoring antiestrogen therapy (e.g., aromatase inhibitor therapy)

- Useful in all applications that require moderately sensitive measurement of E2 including:
  - Evaluation of hypogonadism and oligo-amenorrhea in females
  - Assessing ovarian status, including follicle development, for assisted reproduction protocols (e.g., IVF)

- In conjunction with luteinizing hormone (LH) measurements, monitoring of estrogen replacement therapy in hypogonadal premenopausal women
- Evaluation of feminization, including gynecomastia, in males
- Diagnosis of estrogen-producing neoplasms in males, and, to a lesser degree, females
E2 levels below the premenopausal reference range in young females indicate hypogonadism. If LH and follicle stimulating hormone (FSH) levels are elevated, primary gonadal failure is diagnosed. The main causes are genetic (e.g., Turner syndrome, familial premature ovarian failure), autoimmune (e.g., autoimmune ovarian failure, possibly as part of autoimmune polyglandular endocrine failure syndrome type II), and toxic (e.g., related to chemotherapy or radiation therapy for malignant disease). If LH/FSH levels are low or inappropriately ‘normal,’ a diagnosis of hypogonadotrophic hypogonadism is made. This can have functional causes, such as starvation, overexercise, severe physical or emotional stress, and heavy drug and/or alcohol use. It also can be caused by organic disease of the hypothalamus or pituitary. Further workup is usually necessary, typically including measurement of pituitary hormones (particularly prolactin), and possibly imaging.

Irregular or absent menstrual periods with normal or high E2 levels (and often high E1 levels) are indicative of possible polycystic ovarian syndrome, androgen-producing tumors, or estrogen-producing tumors. Further workup is required and usually includes measurement of total and bioavailable testosterone, androstenedione, dehydroepiandrosterone (sulfate), sex hormone-binding globulin, and possibly imaging.

E2 analysis may be helpful in establishing time of ovulation and optimal time for conception. Optimal time for conception is within 48-72 hours following the midcycle E2 peak. Serial specimens must be collected over several days to evaluate baseline and peak total estrogen (E1 + E2) levels. Low baseline levels and a lack of rise, as well as persistent high levels without midcycle rise, are indicative of anovulatory cycles.

For determining the timing of initiation of ovarian stimulation in IVF studies, low levels (around 30 pg/mL) before stimulation are critical, as higher values often are associated with poor stimulation cycles.

Estrogen replacement in reproductive age women should aim to mimic natural estrogen levels as closely as possible. E2 levels should be within the reference range for premenopausal women, LH/FSH should be within the normal range, and E2 levels should ideally be higher than E1 levels. The current recommendations for postmenopausal female hormone replacement are to administer therapy in the smallest beneficial doses for as briefly as possible. Ideally, E2 and E1 levels should be held below, or near, the lower limit of the premenopausal female reference range.

Postmenopausal women and older men in the lowest quartile of E2 levels are at increased risk of osteoporotic fractures. E2 levels are typically less than 5 pg/mL.

Antiestrogen therapy with central or peripheral acting agents that are not pure receptor antagonists usually aims for complete suppression of E2 production, and in the case of aromatase inhibitors, complete E1 and E2 suppression.

Gynecomastia or other signs of feminization in males may be due to an absolute or relative (in relation to androgens) surplus of estrogens. Gynecomastia is common during puberty in boys. Unless E1, E2, or testosterone levels exceed the adult male reference range, the condition is usually not due to hormonal disease (though it sometimes may still result in persistent breast tissue, which later needs to be surgically removed). For adults with gynecomastia, the workup should include testosterone and adrenal androgen measurements, in addition to E2 and E1 measurements. Causes for increased E1 or E2 levels include:

- High androgen levels caused by tumors or androgen therapy (medical or sport performance enhancing), with secondary elevations in E1 and E2 due to aromatization
- Obesity with increased tissue production of E1
- Decreased E1 and E2 clearance in liver disease
- Estrogen-producing tumors
- Estrogen ingestion

Normal male E1 and E2 levels also may be associated with feminization or gynecomastia, if bioavailable testosterone levels are low due to primary/secondary testicular failure. This may occur, for example, when patients are receiving antiandrogen therapy or other drugs with antiandrogenic effects (e.g., spironolactone, digitalis preparations).

The gonadotrophin-releasing hormone (GnRH) stimulation test remains the central part of the workup for precocious puberty. However, baseline sex steroid and gonadotrophin measurements also are important. Prepubertal girls have E2 levels below 10 pg/mL (most below 5 pg/mL). Levels in prepubertal boys are less than half the levels seen in girls. LH/FSH are very low or undetectable. E1 levels also are low, but may rise slightly, in obese children after onset of adrenarche. E2, which is produced in the gonads, should remain low in these children. In true precocious puberty, both E2 and LH/FSH levels are elevated above the prepubertal range. Elevation of E2 or E1 alone suggests pseudo precocious puberty, possibly due to a sex steroid-producing tumor.

In delayed puberty, estrogens and gonadotrophins are in the prepubertal range. A rise over time predicts the spontaneous onset of puberty. Persistently low estrogens and elevated gonadotrophins suggest primary ovarian failure, while low gonadotrophins suggest hypogonadotropic hypogonadism. In this latter case, Kallman’s syndrome (or related disorders) or hypothalamic/pituitary tumors should be excluded in well-nourished children.

Inherited disorders of sex steroid metabolism are usually associated with production abnormalities of other steroids, most notably a lack of cortisol. Aromatase deficiency is not associated with cortisol abnormalities and usually results in some degree of masculinization in affected females, as well as primary failure of puberty. Males may show delayed (Continued on next page)
Interpretation

**Method**

Estrogens are fractionated into estradiol and estrone by the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The LC-MS/MS method employs an organic extraction to remove water-soluble conjugates and to allow for concentration of the specimen. The method is free from interference and represents a reference methodology.

17 beta-estradiol and estrone are extracted from 0.5 mL of serum with the organic solvent methylene chloride. Deuterated 17 beta-estradiol-d5 and estrone-d4 are added to each specimen before the liquid extraction and serve as internal standards. After derivatization with dansyl chloride, high-pressure liquid chromatography (HPLC) is used prior to introduction of the derivatized sample extract into the tandem mass spectrometer (MS/MS). The calibration utilizes an 8-point standard curve over a concentration range of 0-600 pg/mL. (Anari MR, Bakhtiar R, Zhu B, Huskey, et al: Derivatization of ethynylestradiol with dansyl chloride to enhance electrospray ionization: application in trace analysis of ethynylestradiol in Rhesus monkey plasma. Anal Chem 2002;74,4136-4144)

**Specimen Required:**

Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 1.2 mL of serum refrigerated.

**Note:** Serum must be separated from gel within 24 hours of collection.

**Reference Values:**

ESTRONE (E1)

**Children**

1-14 days: Estrone levels in newborns are very elevated at birth but will fall to prepubertal levels within a few days. Males

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Mean Age</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I#: (&lt;14 days and prepubertal)</td>
<td>7.1</td>
<td>undetectable-16 pg/mL</td>
</tr>
<tr>
<td>Stage II:</td>
<td>11.5</td>
<td>undetectable-22 pg/mL</td>
</tr>
<tr>
<td>Stage III:</td>
<td>13.6</td>
<td>10-25 pg/mL</td>
</tr>
<tr>
<td>Stage IV:</td>
<td>15.1</td>
<td>10-46 pg/mL</td>
</tr>
<tr>
<td>Stage V:</td>
<td>18</td>
<td>10-60 pg/mL</td>
</tr>
</tbody>
</table>

# Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (±2) years. For boys there is no proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

Females

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Mean Age</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I#: (&lt;14 days and prepubertal)</td>
<td>7.1</td>
<td>undetectable-29 pg/mL</td>
</tr>
<tr>
<td>Stage II:</td>
<td>10.5</td>
<td>10-33 pg/mL</td>
</tr>
<tr>
<td>Stage III:</td>
<td>11.6</td>
<td>15-43 pg/mL</td>
</tr>
<tr>
<td>Stage IV:</td>
<td>12.3</td>
<td>16-77 pg/mL</td>
</tr>
<tr>
<td>Stage V:</td>
<td>14.5</td>
<td>17-200 pg/mL</td>
</tr>
</tbody>
</table>

(Continued on next page)
Test Title: Estrogens, Estrone (E1) + Estradiol (E2), Fractionated, Serum #84230

Reference Values:

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for girls at a median age of 10.5 (±2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African-American girls. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

*The reference ranges for children are based on the published literature,1,2 cross-correlation of our assay with assays used to generate the literature data, and on our data for young adults.

Adults

Males 10-60 pg/mL
Females
  Premenopausal: 17-200 pg/mL
  Postmenopausal: 7-40 pg/mL

Conversion factor: E1: pg/mL x 3.704 = pmol/L (molecular wt = 270)

ESTRADIOL (E2)

Children*

1-14 days: Estrone levels in newborns are very elevated at birth but will fall to prepubertal levels within a few days.

Males

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Mean Age</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I#:</td>
<td>7.1</td>
<td>undetectable-13 pg/mL</td>
</tr>
<tr>
<td>(&gt;14 days and prepubertal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II:</td>
<td>10.5</td>
<td>undetectable-24 pg/mL</td>
</tr>
<tr>
<td>Stage III:</td>
<td>11.6</td>
<td>undetectable-60 pg/mL</td>
</tr>
<tr>
<td>Stage IV:</td>
<td>12.3</td>
<td>15-85 pg/mL</td>
</tr>
<tr>
<td>Stage V:</td>
<td>14.5</td>
<td>15-350 pg/mL**</td>
</tr>
</tbody>
</table>

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (±2) years. For boys there is no proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

Females

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Mean Age</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I#:</td>
<td>7.1</td>
<td>undetectable-20 pg/mL</td>
</tr>
<tr>
<td>(&gt;14 days and prepubertal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II:</td>
<td>10.5</td>
<td>undetectable-24 pg/mL</td>
</tr>
<tr>
<td>Stage III:</td>
<td>11.6</td>
<td>undetectable-60 pg/mL</td>
</tr>
<tr>
<td>Stage IV:</td>
<td>12.3</td>
<td>15-85 pg/mL</td>
</tr>
<tr>
<td>Stage V:</td>
<td>14.5</td>
<td>15-350 pg/mL**</td>
</tr>
</tbody>
</table>

#Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for girls at a median age of 10.5 (± 2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African-American girls. Progression through Tanner stages is variable. Tanner stage V (adult) should be reached by age 18.

*The reference ranges for children are based on the published literature,1,2 cross-correlation of our assay with assays used to generate the literature data and on our data for young adults.

Adults

Males 10-40 pg/mL
Females
  Premenopausal: 15-350 pg/mL**
  Postmenopausal: <10 pg/mL

**E2 levels vary widely through the menstrual cycle.

Conversion factor: E2: pg/mL x 3.676 = pmol/L (molecular wt = 272)

Analytic Time: 2 days
Days Set Up: Monday through Friday; 1 p.m.
Fee: $152.00
CPT Code: 82679
82670
NT-Pro B-Type Natriuretic Peptide (BNP), Plasma
#84291

Clinical
B-type natriuretic peptide (brain natriuretic peptide [BNP]) is a small, ringed peptide secreted by the heart to regulate blood pressure and fluid balance. It is stored in and secreted predominantly from membrane granules in the heart ventricles in a pro form (Pro BNP). Once released from the heart in response to ventricle volume expansion and/or pressure overload, the N-terminal (NT) piece of 46 amino acids (NT-Pro BNP) is rapidly cleaved by an enzyme known as corin to release the active 32 amino acid peptide (BNP). Both BNP and NT-Pro BNP are markers of atrial and ventricular distension due to increased intracardiac pressure. The New York Heart Association (NYHA) developed a 4-stage functional classification system for congestive heart failure (CHF) based on the severity of the symptoms. Studies have demonstrated that the measured concentrations of circulating BNP and/or NT-Pro BNP increase with the severity of CHF based on the NYHA classification.

Useful For
Aids in the diagnosis of congestive heart failure.

Interpretation
> normal and <400 pg/mL: likely compensated CHF
≥400 and ≤2,000 pg/mL: likely moderate CHF
>2,000 pg/mL: likely moderate to severe CHF

NT-Pro BNP levels are loosely correlated with NYHA functional class (see Table).

Table: Interpretive Levels for CHF

<table>
<thead>
<tr>
<th>Functional Class</th>
<th>5th to 95th Percentile</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>31-1,110 pg/mL</td>
<td>377 pg/mL</td>
</tr>
<tr>
<td>II</td>
<td>55-4,975 pg/mL</td>
<td>1,223 pg/mL</td>
</tr>
<tr>
<td>III</td>
<td>77-26,916 pg/mL</td>
<td>3,130 pg/mL</td>
</tr>
<tr>
<td>IV</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*In a Mayo study of 75 patients with CHF, only 4 were characterized as Class IV. Accordingly, range and median are not provided.

Cautions
Lack of elevations have been reported if CHF is very acute (first hour) or with ventricular inflow obstruction (hypertrophic obstructive cardiomyopathy, mitral stenosis, atrial myxoma).

Supportive Data
A semiautomated assay for BNP using reagents from Biosite Inc. was introduced at Mayo Medical Laboratories last year (#80022 B-Type Natriuretic Peptide [BNP], Plasma). This automated assay for NT-Pro BNP uses reagents from Roche, Inc. The 2 assays correlate, but the relationship is nonlinear. For normal reference ranges, the Roche NT-Pro BNP levels are 1.5-2.2 times the Biosite BNP levels. With heart failure, the NT-Pro BNP levels are markedly higher compared to BNP levels. The median NT-Pro BNP levels increase much greater with NYHA classification compared to BNP levels, with ratios of 4.0X for class I, 5.5X for class II, 6.8X for class III, and 7.3X for class IV. However, individual patient values vary considerably between these 2 methods, and some patients have NT-Pro BNP values lower than BNP values. The ratios of test values show no consistent relationship with kidney function.

The Roche NT-Pro BNP assay is automated and more precise than the Biosite BNP assay. In addition, in vitro NT-Pro BNP is more stable than BNP. At this time, we are not sure which assay is better for diagnosing and/or monitoring congestive heart failure, so both assays are available. It is recommended that the same assay(s), BNP, NT-Pro BNP, or both, be used for serial measurements.
NT-Pro B-Type Natriuretic Peptide (BNP), Plasma #84291

References

Method
This is an automated double incubation sandwich assay. In the first incubation, antigen from the patient specimen reacts with biotinylated polyclonal sheep NT-Pro BNP antibody and polyclonal NT-Pro BNP antibody labeled with ruthenium complex. During the second incubation, streptavidin-labeled microparticles are added, and the resulting complex is bound to the solid phase via biotin-streptavidin interaction. The resulting reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of an electrode. Unbound substances are removed with ProCell. Voltage is then applied to the electrode, and this induces chemiluminescent emission that is measured by a photomultiplier. Results are obtained by comparing this measurement against the calibration curve. (Roche Pro BNP Test Product Insert. Roche Diagnostics Corporation, Indianapolis, IN, Jan 2003)

Specimen Required: Draw blood in a lavender-top (EDTA) tube(s). Spin down and send 0.5 mL of EDTA plasma.

Reference Values:

<table>
<thead>
<tr>
<th>Males:</th>
<th>Females:</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤45 years: ≤51 pg/mL</td>
<td>46 years: ≤140 pg/mL</td>
</tr>
<tr>
<td>46 years: ≤53 pg/mL</td>
<td>59 years: ≤171 pg/mL</td>
</tr>
<tr>
<td>47 years: ≤55 pg/mL</td>
<td>71 years: ≤103 pg/mL</td>
</tr>
<tr>
<td>48 years: ≤56 pg/mL</td>
<td>59 years: ≤173 pg/mL</td>
</tr>
<tr>
<td>49 years: ≤58 pg/mL</td>
<td>72 years: ≤104 pg/mL</td>
</tr>
<tr>
<td>50 years: ≤59 pg/mL</td>
<td>60 years: ≤107 pg/mL</td>
</tr>
<tr>
<td>51 years: ≤61 pg/mL</td>
<td>73 years: ≤110 pg/mL</td>
</tr>
<tr>
<td>52 years: ≤62 pg/mL</td>
<td>61 years: ≤113 pg/mL</td>
</tr>
<tr>
<td>53 years: ≤64 pg/mL</td>
<td>74 years: ≤116 pg/mL</td>
</tr>
<tr>
<td>54 years: ≤67 pg/mL</td>
<td>62 years: ≤119 pg/mL</td>
</tr>
<tr>
<td>55 years: ≤68 pg/mL</td>
<td>75 years: ≤122 pg/mL</td>
</tr>
<tr>
<td>56 years: ≤70 pg/mL</td>
<td>76 years: ≤125 pg/mL</td>
</tr>
<tr>
<td>57 years: ≤71 pg/mL</td>
<td>77 years: ≤128 pg/mL</td>
</tr>
<tr>
<td>58 years: ≤73 pg/mL</td>
<td>78 years: ≤131 pg/mL</td>
</tr>
<tr>
<td>59 years: ≤76 pg/mL</td>
<td>79 years: ≤135 pg/mL</td>
</tr>
<tr>
<td>60 years: ≤77 pg/mL</td>
<td>80 years: ≤138 pg/mL</td>
</tr>
<tr>
<td>61 years: ≤79 pg/mL</td>
<td>81 years: ≤141 pg/mL</td>
</tr>
<tr>
<td>62 years: ≤82 pg/mL</td>
<td>82 years: ≤144 pg/mL</td>
</tr>
<tr>
<td>63 years: ≤83 pg/mL</td>
<td>83 years: ≤146 pg/mL</td>
</tr>
<tr>
<td>64 years: ≤85 pg/mL</td>
<td>65 years: ≤149 pg/mL</td>
</tr>
<tr>
<td>65 years: ≤88 pg/mL</td>
<td>71 years: ≤150 pg/mL</td>
</tr>
<tr>
<td>66 years: ≤89 pg/mL</td>
<td>52 years: ≤152 pg/mL</td>
</tr>
<tr>
<td>67 years: ≤92 pg/mL</td>
<td>65 years: ≤155 pg/mL</td>
</tr>
<tr>
<td>68 years: ≤95 pg/mL</td>
<td>66 years: ≤157 pg/mL</td>
</tr>
<tr>
<td>69 years: ≤97 pg/mL</td>
<td>67 years: ≤160 pg/mL</td>
</tr>
<tr>
<td>70 years: ≤100 pg/mL</td>
<td>68 years: ≤162 pg/mL</td>
</tr>
</tbody>
</table>

Analytic Time: Same day/1 day
Days Set Up: Monday through Sunday; Continuously
Fee: $134
CPT Code: 83880
Clinical

Ehrlichiosis is a group of emerging zoonotic infections caused by *Anaplasma* and *Ehrlichia* species, which are obligate intracellular, gram-negative rickettsial organisms that infect human leukocytes.

Human granulocytic ehrlichiosis (HGE) is caused by the tick-borne rickettsia, *Anaplasma phagocytophila*, which is transmitted by contact with *Ixodes* ticks. The deer mouse is the animal reservoir, and the epidemiology of this infection is very much like that of Lyme disease (caused by *Borrelia burgdorferi*) and babesiosis (caused by *Babesia microti*), which have the same tick vector as human ehrlichiosis (HE). HGE is most prevalent in the upper Midwest and in other areas of the United States (US) that are endemic for Lyme disease.

Febrile illnesses accompanied by granulocytic cytoplasmic inclusions (morulae) in patients are suggestive of ehrlichial infection. Selective infection of granulocytes results in an acute febrile illness following tick exposure and may include laboratory findings of leukopenia or thrombocytopenia. However, these latter findings also may be present in patients with Lyme disease or babesiosis. Ticks coinfect with both *Borrelia* and *Ehrlichia* and *Ehrlichia* may transmit both pathogens.

Human monocytic ehrlichiosis (HME) is caused by *Ehrlichia chaffeensis*, which is transmitted by the Lone Star tick, *Amblyomma americanum*. The deer is believed to be the animal reservoir, and most cases of HME have been reported from the southeastern and south-central regions of the US.

*Ehrlichia ewingii*, the known cause of canine granulocytic ehrlichiosis, can occasionally cause an HME-like illness in humans. Clinical features and laboratory abnormalities are similar to those of *Ehrlichia chaffeensis* infection, and antibodies to *Ehrlichia ewingii* cross-react with current serologic assays for detection of antibodies to *Ehrlichia chaffeensis*.

Infecive forms of the rickettsial organisms are injected during tick bites and the organisms enter the vascular system where they infect leukocytes. They are sequestered in host-cell membrane-limited parasitophorous vacuoles known as morulae. These morulae can be readily observed on Giemsa- or Wright's-stained smears of peripheral blood from infected persons. Macrophages in organs of the reticuloendothelial system also are infected. Asexual reproduction occurs in white blood cells (WBCs) where daughter cells are formed and liberated upon rupture of the WBCs.

Most cases of ehrlichiosis are probably subclinical or mild, but the infection can be severe and life-threatening with a 2-3% mortality rate. Fever, fatigue, malaise, headache, and other "flu-like" symptoms including myalgias, arthralgias, and nausea, occur most commonly. Central nervous system involvement can result in seizures and coma.

Diagnosis of HE has been difficult because the patient's clinical course is often mild and nonspecific. This symptom complex is easily confused with other illnesses such as influenza, or other tick-borne zoonoses such as Lyme disease, babesiosis, and Rocky Mountain spotted fever. Clues to the diagnosis of ehrlichiosis in an acutely febrile patient after tick exposure include laboratory findings of leukopenia or thrombocytopenia and elevated serum aminotransferase levels. However, while these abnormal laboratory findings are frequently seen, they are not specific. PCR techniques allow direct detection of pathogen-specific DNA from patients' whole blood during the acute phase of disease. Serologic testing is usually done only for confirmatory purposes, by demonstrating a 4-fold rise or fall in specific antibody titers to *Ehrlichia* or *Anaplasma* antigens.

Useful For

Evaluating patients suspected of HGE or HME
**Test Title:** Ehrlichia/Anaplasma DNA Detection by Rapid Polymerase Chain Reaction (PCR), Blood

#84319

**Interpretation**
Positive results indicate the presence of specific DNA from *Ehrlichia chaffeensis*, *Ehrlichia ewingii*/*Ehrlichia canis*, or *Anaplasma phagocytophila*, and support the diagnosis of HE.

Negative results indicate the absence of detectable DNA from any of these pathogens in the specimen, but it does not negate the presence of the organism and/or active/recent disease.

Since DNA of *Ehrlichia ewingii* is indistinguishable from that of *Ehrlichia canis* by this rapid PCR assay, a positive result for *Ehrlichia ewingii*/*Ehrlichia canis* indicates the presence of DNA from either of these 2 organisms.

**Cautions**
- A negative result does not indicate absence of disease.
- Inhibitory substances may be present in the patient’s whole blood specimen.
- Inadequate specimen draw or improper conditions for storage and/or transport may invalidate test results.
- This test may detect DNA of *Ehrlichia canis* (reported to cause asymptomatic infection in Venezuela only) and *Ehrlichia muris* (which has not been reported to cause human infections).
- This PCR test does not detect DNA of *Ehrlichia senettenu*, which has been reported to cause a rare mononucleosis-like illness in humans (in Japan and Malaysia).

**References**

**Method**
Nucleic acid is extracted from the pathogens in blood using the automated MagNA Pure LC instrument system. The extract is then transferred to individual self-contained capillary cuvettes for amplification. The LightCycler is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of PCR. The DNA target for PCR assay is groEL, the open reading frame gene segment of the heat-shock protein operon (groESL), which is present at a frequency of 1 copy per organism in pathogenic species of *Anaplasma* and *Ehrlichia*. A specific base pair DNA target sequence is amplified by PCR. The detection of amplicon is based on fluorescence resonance energy transfer (FRET), which utilizes a hybridization probe with a donor fluorophore, fluorescein, at the 3’ end, and an acceptor fluorophore, LC-Red 640, at the 5’ end. When the target amplicon is present, the LC-Red 640 emits a measurable and quantifiable light signal at a specific wavelength. Presence of the specific organism nucleic acid may be confirmed by performing a melting curve analysis of the amplicon. Using features of the melting curve analysis, the assay printers and specific hybridization probes are able to detect and differentiate among *Anaplasma phagocytophila* (Tm=66.5 degrees C), *Ehrlichia chaffeensis* (Tm=59.2 degrees C), and *Ehrlichia ewingii*/*Ehrlichia canis* (Tm=50.2 / 49.5 degrees C). Due to close proximity of the melting curves of *Ehrlichia ewingii* and *Ehrlichia canis*, this assay cannot distinguish between these 2 organisms. (Cockerill FR, Uhl FR: Applications and challenges of real-time PCR for the clinical microbiology laboratory. In Rapid Cycle Real-Time PCR. Edited by U Reischl, C Wittwer, F Cockerill. Springer, NY 2002)

**Specimen Required:** Draw blood in lavender-top (EDTA) tube(s), and send 5.0 mL of EDTA whole blood refrigerated. Maintain sterility and forward promptly.

**Note:** If ordering electronically, no form is required with the specimen. If not ordering electronically, please complete and submit a “Microbiology Request Form” (Supply T244) with the specimen.

**Reference Values:** Negative

**Analytic Time:** Same day / 1 day

**Days Set Up:** Monday through Friday; 12 p.m.

**Fee:** $211.90

**CPT Code:** 87798
Brucella Antibody, IgG & IgM, Serum
#84327

Clinical
Worldwide, brucellosis remains a major disease in humans and domesticated animals. Brucella infects goats (Brucella melitensis), cattle (Brucella abortus), swine (Brucella suis), and dogs (Brucella canis). The disease has a limited geographic distribution. Few cases occur in the United States, with the bulk occurring in the Mediterranean region, Western Asia, and parts of Latin America and Africa.

Three species of Brucella commonly cause disease in humans: Brucella melitensis, Brucella suis, and Brucella abortus. The acute disease often presents with fever, chills, and malaise; the chronic form also causes abscesses in bone, brain, spleen, liver, and kidney.

Useful for
Evaluating patients with suspected brucellosis

Interpretation
In the acute stage of the disease there is the initial production of IgM antibodies, followed closely by IgG. IgG antibodies may decline rapidly after treatment; however, high levels of circulating IgG antibodies may be found without any active disease. Chronic brucellosis shows predominance if IgG antibodies with little or no IgM present.

Rising levels of specific antibody in paired sera can be regarded as serological evidence of recent infection. The presence of specific IgM in a single specimen may indicate a recent infection. A positive result in a single specimen is suggestive of recent infection or a presumptive diagnosis in the presence of a supporting clinical presentation.

If specific IgM and IgG antibodies are not detected and a recent infection is suspected, this can be confirmed by testing a further specimen 7-14 days later.

Cautions
Brucella canis, a rare cause of brucellosis, may not be detected by this method. Any laboratory results (especially serology) should be interpreted in conjunction with other laboratory and clinical findings.

Supportive Data
A total of 92 serum specimens were evaluated for the presence of Brucella IgG antibodies using a tube agglutination method and this IgG/IgM ELISA test. The result for the ELISA method was considered positive if either or both the IgG and IgM was positive. The result for the agglutination method was considered positive if the titer was \( \geq 1:80 \). The sensitivity of the ELISA method compared to the agglutination method as the "gold standard" was 64.7% (specificity 93.1%). Medical histories were reviewed to reconcile discordant results. For 1 of the patients, it could not be determined if the patient had active or past disease and this patient was eliminated from the final analysis. For all other discordant results, an assessment for disease (current or past) was possibly by chart review. The corrected sensitivity and specificity for the ELISA method was 100% and 97%, respectively. This analysis revealed that lower titer positive results (\( \leq 1:160 \)) for the agglutination method were frequently false-positive results; these low titer-positive specimens were negative by the ELISA method.
Test Title: Brucella Antibody, IgG & IgM, Serum
#84327

References

Method
Serum is tested using ELISA. Serum antibodies, when present combine with Brucella antigen attached to the polystyrene surface of the microwells. Residual serum is removed by washing and peroxidase conjugated antihuman IgG or IgM is added. The microwells are washed and a colorless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB/H[2]O[2]) is added. The substrate is hydrolyzed by the enzyme and the chromogen changes to a blue color. After stopping the reaction with acid, the TMB becomes yellow. The color intensity is directly related to the concentration of the Brucella IgG antibodies in the test specimen. The absorbance of each well is read at a wavelength of 450 nm. (Package inserts: Brucella IgG ELISA test/Brucella IgM ELISA test, PanBio, Inc,. Columbia, MD)

Specimen Required: Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 1.0 mL of serum refrigerated in a screw-capped, plastic vial.

Reference Values:
IgG:
- Negative (Reported as positive, negative, or equivocal)
IgM:
- Negative (Reported as positive, negative, or equivocal)

Analytic Time:
Same day/1 day

Days Set Up:
Monday through Saturday; 10:00 a.m.

Fee:
$88.80

CPT Code:
86622\x2
Hepatitis C Virus (HCV) Qualitative (with Reflex to HCV Quantitation by bDNA), Serum #84337

Profile Information

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Reporting Name</th>
<th>Available Separately</th>
</tr>
</thead>
<tbody>
<tr>
<td>81130</td>
<td>HCV QN by bDNA, S</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Clinical

Monitoring patients with hepatitis C virus (HCV) infection requires determination of both qualitative and quantitative HCV levels. Because qualitative measures are more sensitive than quantitative measures, qualitative tests are typically ordered first. More importantly, the more sensitive qualitative test is used to monitor therapy; treatment is often discontinued in nonresponders (i.e., qualitative HCV test remains positive during treatment). The quantitative test is used to determine the length of therapy, with higher levels requiring longer treatment.

See "Advances in the Laboratory Diagnosis of Hepatitis C" in Publications. Also see the update, "Hepatitis C Virus Algorithm Changes."

Useful for

- Determination of quantitative HCV RNA titer when the qualitative HCV RNA result is positive
- Confirmation of presence of HCV RNA before testing for quantitative HCV RNA titer
- Monitoring suppression of HCV replication during anti-HCV therapy

Interpretation

In this ordering option, a positive qualitative HCV reverse transcription-polymerase chain reaction (RT-PCR) test reflexes to the HCV bDNA quantitative test and an HCV RNA titer value is reported.

A positive qualitative test result indicates the presence of HCV RNA. The lower limit of detection of the qualitative RT-PCR test is 50 IU/mL.

For a specimen with detectable HCV RNA by qualitative RT-PCR test, the subsequent quantitative test by bDNA may yield a HCV RNA titer in the range of 615-7,690,000 IU/mL (the quantitative reportable range of the bDNA assay).

A quantitative result of <615 IU/mL in a specimen with a positive qualitative HCV RNA result indicates that the HCV RNA titer is below the lower limit of detection of the quantitative test and is somewhere in the range of 50-614 IU/mL.

A quantitative result of >7,690,000 IU/mL indicates that the HCV RNA titer is above the upper limit of detection of the quantitative test.

Cautions

Positive qualitative and/or quantitative HCV RNA test results should not be used as the only criterion for the diagnosis of acute or chronic HCV infection. Results must be interpreted in the context of patient’s symptoms, clinical presentation, and anti-HCV antibody serologic test result.
Test Title: Hepatitis C Virus (HCV) Qualitative (with Reflex to HCV Quantitation by bDNA), Serum #84337

References

Method
This COBAS AMPLICOR HCV Test, v2.0 is a Food and Drug Administration (FDA)-approved test. To perform the RT-PCR procedure, a solvent extraction protocol is used to isolate RNA free from inhibitors that are often present in the specimen. A specific RNA target sequence encoding a portion of the highly conserved 5’ untranslated region of HCV is converted to complementary DNA (cDNA) and then amplified up to 10(12)-fold by PCR.

This test is based on 4 major processes performed on extracted RNA from serum: RT of target RNA to generate cDNA, PCR target amplification, hybridization of the amplified product to a specific oligonucleotide probe, and detection of the probe-bound amplified product by color formation.

The COBAS AMPLICOR HCV Test, v2.0 FDA-approved test is able to detect 50 IU/mL from both serum and plasma specimens. (Poljak M, Sene K, Koren S: Evaluation of the automated COBAS AMPLICOR hepatitis C virus PCR system. J Clin Microbiol 1997;35:2983-2984)

The VERSANT HCV RNA 3.0 Assay is a sandwich nucleic acid hybridization procedure for the quantification of HCV RNA in human serum and plasma. In an overnight incubation, HCV RNA is liberated from virions, and its capture to a microwell is mediated by a set of specific, synthetic oligonucleotide capture probes (capture extenders). A set of target probes (label extenders) also is hybridized to the viral RNA during this incubation step. The 2 sets of target probes bind to the 5’-untranslated and core regions of the HCV genome. These target probes have been specifically designed to capture and quantify HCV genotypes 1-6 equally.

After the overnight incubation, the preamplifier and amplifier probes are hybridized to the target probes (label extenders). Multiple copies of an alkaline phosphatase-conjugated probe (label probe) are then hybridized to the immobilized complex. Detection is achieved by incubating the complex with a chemiluminescent substrate and measuring the light emission generated by the bound alkaline phosphatase reacting with the chemiluminescent substrate. Light emission is directly proportional to the amount of HCV RNA present in each specimen. Results are recorded as relative light units by the System 340 Analyzer. A standard curve is defined by light emission from 5 standards containing known concentrations of recombinant bacteriophage. Concentrations of HCV RNA in specimens are then determined from this standard curve. (Germer JJ, Heimgartner PJ, Istrup DM, et al: Comparative Evaluation COBAS AMPLICOR HCV MONITOR Version 2.0 Assay for the Quantification of the VERSANT HCV RNA 3.0, Quantiplex HCV RNA 2.0, and of Hepatitis C Virus RNA in Serum. J Clin Microbiol 2002;40:495-500.)

Specimen Required: Draw blood in a serum gel tube(s). Aseptically spin down within 4 hours of draw and send 1.0 mL of serum frozen in a screw-capped, sterile, plastic vial. Maintain sterility and forward promptly.

Reference Values: Negative for HCV-RNA

Analytic Time: 2 to 4 days
Days Set Up: Monday through Sunday; 10:00 a.m.
Fee: $271.30
CPT Code: 87521/HCV, Qualitative
87522/HCV, Quantitative (if appropriate)
Severe Acute Respiratory Syndrome (SARS) Antibody IgG, Serum #84339

Clinical

An outbreak of atypical pneumonia, (severe acute respiratory syndrome [SARS]) was first identified in Guangdong Province, China in late 2002. A unique coronavirus (SARS-CoV) was determined to be the etiologic agent by recovery in cell cultures, examination of morphology by electronic microscopic (EM), serologic antibody determinations, and molecular amplification and sequencing of target RNA of the agent.

The Coronaviridae family contains 2 genera: torovirus and coronavirus. The toroviruses, recognized pathogens of horses and cattle, also have been detected in some gastrointestinal infections in humans. Their role in the etiology of human infection has not been established, although these viruses have been associated with diarrhea in children. In addition to human infections, coronaviruses infect several animal species; infectious bronchitis virus from chickens with respiratory disease and transmissible gastroenteritis virus in swine are notable examples. SARS-CoV has also been recovered from cats and ferrets.

Coronaviruses have unique morphologic features by EM analysis; viral particles are 100-150 nm in diameter surrounded by club-faced crown-appearing projections on the surface representing the spike proteins. The virus acquires a lipid membrane, presumably required for the ability to infect new cells, as it buds from the membranes of the Golgi apparatus or endoplasmic reticulum of the host cell. The single strand of RNA contains approximately 30 kb.

Clinically, the incubation period for SARS-CoV infection in humans is approximately 2-10 days. Early manifestations include influenza-like symptoms, such as fever, myalgias, and headache. The respiratory phase starts within 2-4 days of onset of fever with a dry, nonproductive cough. The case fatality rate is approximately 3-12% overall. The mortality rate may be as high as 45% in patients older than 60 years, particularly those with preexisting comorbidity (e.g., diabetes, renal failure, and other chronic conditions).

Useful for

To aid in the diagnosis of SARS

Interpretation

Because of the emergence of this new strain of SARS-CoV, antibodies to this virus will be limited (in background prevalence) to those geographical areas in which outbreaks were clearly documented during 2002 and 2003 (China, Canada, and 30 other countries).

The presence of IgG antibodies very likely reflects recent primary infection with the virus. Antibodies to SARS-CoV become detectable around 10 days after infection. A negative serologic result 21 days after onset of symptoms suggests absence of SARS-CoV infection.

If patient has a syndrome suggestive of or consistent with SARS, contact your local infection control service and local public health authority for further advice.

Cautions

- Use of heat-inactivated serum or plasma is not recommended as this may lead to false-positive results.
- Diagnosis of SARS relies on the exclusion of other viral and bacterial causes of respiratory tract disease.
- The kit is for research use only. Not for use in diagnostic procedures.
**Test Title:** Severe Acute Respiratory Syndrome (SARS) Antibody IgG, Serum #84339

**Method**

Serum samples are added to wells coated with SARS-CoV-derived antigens. If antibodies specific for SARS-CoV are present in the sample, they form stable complexes with the SARS-CoV antigens attached to the well. The microplate wells are washed and a goat IgG or IgM antihuman immunoglobulin labeled with horseradish peroxidase is added. If the antigen/antibody complex is present, the peroxidase conjugate binds to the complex and remains in the well. After a second wash step, enzyme substrate is added. During incubation, a blue color develops in proportion to the amount of anti-SARS-CoV antibodies bound to the well. Wells containing samples negative for anti-SARS-CoV antibody remain colorless. An acid stop solution is added to each well and the absorbance read on a microplate reader at 450 nm.

If the initial absorbance value indicates a positive result, the original sample from the patient is tested in duplicate. If 1 or both retest values are reactive, then the sample is considered to yield a positive result for IgG antibodies to SARS-CoV. (Package insert: DETECT-SARS. Adaltis Inc, Montreal, Quebec, Canada.)

**References**


**Specimen Required:** Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 0.5 mL of serum refrigerated.

**Reference Values:** Negative

**Analytic Time:** Same day/1 day

**Days Set Up:** Monday through Saturday; Varies

**Fee:** $109.00

**Test Classification:** FOR RESEARCH USE ONLY. Performance characteristics have been determined by Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN. Results should be interpreted in conjunction with clinical findings.

**CPT Code:** 86790
Parathyroid Hormone, 1-84 Bio-Intact with Minerals, Serum
#84341

If you do not require mineral calculation, please see New Test Announcement #84213 Parathyroid Hormone, 1-84 Bio-Intact, Serum.

Clinical

Parathyroid hormone (PTH) is produced and secreted by the parathyroid glands located along the posterior aspect of the thyroid gland. The hormone is synthesized as a 115-amino acid precursor (pre-pro-PTH), cleaved to pro-PTH and then to the 84-amino acid molecule, PTH (numbering, by universal convention, starting at the amino-terminus). The precursor forms generally remain within the parathyroid cells. Secreted PTH undergoes cleavage and metabolism to form carboxyl-terminal fragments (PTH-C), amino-terminal fragments (PTH-N), and midmolecule fragments (PTH-M). Only those portions of the molecule that carry the aminoterminus (i.e., the whole molecule and PTH-N) are biologically active. The active forms have half-lives of approximately 5 minutes. The inactive PTH-C fragments, with half-lives of 24-36 hours, make up >90% of the total circulating PTH and are primarily cleared by the kidneys. In patients with renal failure, they can accumulate to very high levels. Unlike many other PTH immunoassays, the 1-84 Bio-Intact PTH assay measures exclusively the biologically active whole PTH molecule (1-84) and does not cross-react with any PTH fragments. This facilitates the correct diagnosis of parathyroid disorders, particularly in patients with renal failure.

The serum calcium level regulates PTH secretion via negative feedback through the parathyroid calcium sensing receptor (CASR). Decreased calcium levels stimulate PTH release. Secreted PTH interacts with its specific type II G-protein receptor, causing rapid increases in renal tubular reabsorption of calcium and decreased phosphorus reabsorption. It also participates in long-term calciostatic functions by enhancing mobilization of calcium from bone and increasing renal synthesis of 1,25-dihydroxy vitamin D, which, in turn, increases intestinal calcium absorption. In rare inherited syndromes of parathyroid hormone resistance/unresponsiveness and in renal failure, PTH release may not increase serum calcium levels.

Hyperparathyroidism causes hypercalcemia, hypophosphatemia, hypercalcuria, and hyperphosphaturia. Long-term consequences are dehydration, renal stones, hypertension, GI disturbances, osteoporosis, and sometimes neuropsychiatric and neuromuscular problems. Hyperparathyroidism is most commonly primary and caused by parathyroid adenomas. It can also be secondary in response to hypocalcemia or hyperphosphatemia. This is most commonly observed in renal failure. Long-standing secondary hyperparathyroidism can result in tertiary hyperparathyroidism, which represents the secondary development of autonomous parathyroid hypersecretion. Rare cases of mild, benign hyperparathyroidism can be caused by inactivating CASR mutations.

Hypoparathyroidism is most commonly secondary to thyroid surgery, but can also occur on an autoimmune basis, or due to activating CASR mutations. The symptoms of hypoparathyroidism are primarily those of hypocalcemia, with weakness, tetany, and possible optic nerve atrophy.

Useful for
• Diagnosis and differential diagnosis of hypercalcemia
• Diagnosis of primary, secondary, and tertiary hyperparathyroidism
• Diagnosis of hypoparathyroidism
• Differential diagnosis of hypercalcemia and renal osteodystrophy
Interpretation

• About 90% of the patients with primary hyperparathyroidism have elevated PTH levels. The remaining patients have normal (inappropriate for the elevated calcium level) PTH levels. About 40% of the patients with primary hyperparathyroidism have serum phosphorus levels <2.5 mg/dL and about 80% have serum phosphorus <3.0 mg/dL.
• Some patients with moderate hypercalcemia and equivocal phosphate levels, who have either mild elevations in PTH or (inappropriately) normal PTH levels, may be suffering from familial hypocalciuric hypercalcemia, which is due to inactivating CASR mutations. The renal calcium clearance to creatinine clearance ratio is typically less than 0.01 in these individuals. The condition can be confirmed by CASR gene mutation screening.
• An (appropriately) low PTH level and high phosphorus level in a hypercalcemic patient suggests that the hypercalcemia is not caused by PTH or PTH-like substances.
• An (appropriately) low PTH level with a low phosphorus level in a hypercalcemic patient suggests the diagnosis of paraneoplastic hypercalcemia caused by parathyroid related peptide (PTHRP). PTHRP shares N-terminal homology with PTH and can transactivate the PTH receptor. It can be produced by many different tumor types.
• A low or normal PTH in a patient with hypocalcemia suggests hypoparathyroidism, provided the serum magnesium level is normal. Low magnesium levels inhibit PTH release and action and can mimic hypoparathyroidism.
• Low serum calcium and high PTH levels in a patient with normal renal function suggest resistance to PTH action (pseudohypoparathyroidism type 1a, 1b, 1c, or 2) or, very rarely, bio-ineffective PTH.
• Patients with renal failure, whose serum phosphate levels are normal, but who still have elevated Bio-Intact PTH (1-84) levels, probably suffer from secondary or tertiary hyperparathyroidism. If their corrected or ionized serum calcium is normal or high, then the most likely diagnosis is tertiary hyperparathyroidism.

Cautions

• For diagnostic purposes, Bio-Intact PTH (1-84) values should be interpreted with other test results, and the overall clinical presentation and history of the patient.
• Normal reference ranges may vary based on geographical locations of the populations studied.
• Although the PTH-N fragments PTH(1-31) or PTH(1-34) do not cross-react in the assay, as they do not bind to both detection and capture antibodies, they do bind to 1 of the assay’s antibodies. Therefore, if PTH(1-31) or PTH(1-34) are present at very high concentrations, they may bind sufficient antibody to interfere in the assay, resulting in falsely low Bio-Intact PTH (1-84) measurements. There are no known disease states where this could occur. However, this situation could conceivably be seen when a patient is receiving exogenous PTH(1-34). Exercise caution when interpreting PTH levels in these patients.

References

Method

The Nichols Advantage Bio-Intact (1-84) assay is a 2-site chemiluminescence immunoassay for the measurement of biologically active, whole molecule parathyroid hormone (PTH) in human serum. The patient specimen is incubated simultaneously with a biotinylated-goat polyclonal antibody to PTH, a second goat polyclonal antibody to PTH labeled with an acridinium ester, and streptavidin coated magnetic particles. During the subsequent 30-minute incubation, a sandwich complex is formed by the 2 antibodies and PTH in the patients' specimen. This complex is captured by the streptavidin-coated particles due to high affinity interaction between biotin and streptavidin. The magnetic particles are washed to remove any unbound components and then transported into a luminometer where a chemiluminescence reaction is initiated. The amount of light generated is directly proportional to the concentration of PTH in the specimen. (Package Insert: Nichols Advantage Bio-Intact PTH (1-84). Nichols Institute Diagnostics, San Juan Capistrano, CA)

Specimen Required:
Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down, separate from clot, and send 0.8 mL of serum frozen in plastic vial.

Reference Values:

PTH, 1-84 BIO-INTACT
<2 years: Not established
≥2 years: 10-55 pg/mL
≥2 years: 1.1-5.8 pmol/L

Conversion factor: PTH: pg/mL x 0.1061 = pmol/L (molecular wt = 9424)

The reference range has been generated from healthy US Northern Latitude donors. The upper limit of the reference range is likely to be lower in geographical regions with greater sunlight-exposure. In the presence of normal renal function and hypercalcemia, a PTH, 1-84 Bio-Intact level of >35 pg/mL is highly suggestive of primary hyperparathyroidism.

CALCIUM
Males
0-11 months: not established
1-14 years: 9.6-10.6 mg/dL
15-16 years: 9.5-10.5 mg/dL
17-18 years: 9.5-10.4 mg/dL
19-21 years: 9.3-10.3 mg/dL
≥22 years: 8.9-10.1 mg/dL
Females
0-11 months: not established
1-11 years: 9.6-10.6 mg/dL
12-14 years: 9.5-10.4 mg/dL
15-18 years: 9.1-10.3 mg/dL
≥19 years: 8.9-10.1 mg/dL

(Reference values continued on next page)
Reference Values:

PHOSPHORUS

Males
- 0-11 months: not established
- 1-4 years: 4.3-5.4 mg/dL
- 5-13 years: 3.7-5.4 mg/dL
- 14-15 years: 3.5-5.3 mg/dL
- 16-17 years: 3.1-4.7 mg/dL
- ≥18 years: 2.5-4.5 mg/dL

Females
- 0-11 months: not established
- 1-7 years: 4.3-5.4 mg/dL
- 8-13 years: 4.0-5.2 mg/dL
- 14-15 years: 3.5-4.9 mg/dL
- 16-17 years: 3.1-4.7 mg/dL
- ≥18 years: 2.5-4.5 mg/dL

CREATININE

Males:
- 0-11 months: not established
- 1-2 years: 0.2-0.6 mg/dL
- 3-4 years: 0.3-0.7 mg/dL
- 5-9 years: 0.4-0.8 mg/dL
- 10-11 years: 0.5-0.9 mg/dL
- 12-13 years: 0.6-1.0 mg/dL
- 14-15 years: 0.7-1.1 mg/dL
- ≥16 years: 0.9-1.4 mg/dL

Females:
- 0-11 months: not established
- 1-3 years: 0.3-0.6 mg/dL
- 4-5 years: 0.4-0.7 mg/dL
- 6-8 years: 0.5-0.8 mg/dL
- 9-15 years: 0.6-0.9 mg/dL
- ≥16 years: 0.7-1.2 mg/dL

Test Title: Parathyroid Hormone, 1-84 Bio-Intact with Minerals, Serum
#84341

Analytic Time: 1 day
Days Set Up: Monday through Saturday; 8 a.m. - 2 p.m.
Fee: $201.10
CPT Code: 82310/Calcium
82565/Creatinine
83970/PTH
84100/Phosphorus